

BREAKING SEED DORMANCY IN *TAMARINDUS INDICA* USING DIFFERENT PRE-TREATMENT METHODS

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ABSTRACT

This study investigated the germination and seedling growth of *Tamarindus indica*, focusing on overcoming seed dormancy to enhance its sustainable propagation and utilization. As a highly valued tropical species, *T. indica* offers significant ecological, medicinal, and industrial benefits but faces challenges such as habitat loss and dormancy-related low germination rates. Using seeds obtained from Kaduna, Nigeria, the research applied four dormancy-breaking methods: normal water soaking, hot water treatment, mechanical scarification, and varying concentrations of sulfuric acid. Germination performance and early growth were assessed under controlled conditions using germination incubators and polythene bag setups with potting mixtures. Phytochemical analyses revealed the presence of important secondary metabolites, including flavonoids, tannins, alkaloids, and saponins, emphasizing the plant's medicinal potential. The results showed significant ($p < 0.05$) variations in germination and seedling vigor among the treatments, with sulfuric acid demonstrating the highest efficacy in breaking dormancy and promoting uniform germination. These findings show the importance of selecting appropriate dormancy-breaking methods for enhancing the cultivation of *T. indica*. The study presents the need for conservation and sustainable agricultural practices to prevent the extinction of this valuable species while promoting its industrial and ecological utility. This research contributes critical insights for afforestation programs, pharmaceutical raw material production, and biodiversity conservation, ensuring the long-term availability of *T. indica* for future generations.

Keywords: *Tamarindus indica*, phytochemical analysis, dormancy, germination, seedling.

INTRODUCTION

Tamarind is a multipurpose plant. The pulp of the fruit has been used as a spice in Asian cuisine, especially in the southern part of India, for a long time. Almost all parts of the tree are used in the food, chemical, pharmaceutical or textile industries, or as fodder, timber and fuel (Soni *et al.*, 2023).

Tamarindus indica; which is one of the highly commercialized medicinal plants is known for its potent anti-inflammatory activities. This tropical tree has been used to treat inflammation, stomach pain, throat pain and rheumatism in traditional medicine. Additionally, the plant has also been used to manage myriads of other disease conditions including wound healing, diarrhea, dysentery, parasitic infestation, fever, malaria etc., and also highly valued as food supplement. In fact, the medicinal activities and use of *T. indica* in traditional folk medicine are attributed to the presence of phytochemicals in the different part of the plant including flavonoids, alkaloids, tannins, phenols, triterpenoids, fatty acids, saponins and steroids (Richard *et al.*, 2019).

T. indica in Nigeria have been exploited more or less as wild form. The aging tree stands are gradually dying without replacements and seeds do not germinate of their own, possibly due to lack of the factor that is required to break the dormancy. To enhance rapid sustainable production of *T. indica* there is a need for an understanding of the basic silvicultural requirements of the species. Knowledge on seed germination is known to be an important tool for any afforestation success. In addition, the role of *T. indica* in environmental protection cannot be over emphasized (Richard *et al.*, 2019).

T. indica is an indigenous legume, which has been recognized as a potential nitrogen fixing tree in the semi-arid region of Nigeria (Muhammad and Amusa 2003). *T. indica* is a tree in the family fabaceae native to tropical Africa. The genus is a monotypic taxon, having only a single species. It is cultivated widely in India, Bahamas, West Indies, and USA among others. The species is a slow-growing one; long lived massive tree reaches under favourable condition, a height of 12–24 m and a trunk circumference of 7.5 m. The bright green, fine feathery foliage is composed of pinnate leaves, each having 10–15 pairs of oblong leaflets 1.25–2.5 cm long and 5–6 mm wide which fold at night. It is one of the most widespread trees of the Indian subcontinent. It is a large evergreen tree with an exceptionally beautiful spreading crown, and is cultivated throughout the whole of India, except in the Himalayas and western dry regions (Pugalenthi *et al.*, 2004).

In Nigeria the growth and management of Tamarind are done by local farmers. Nursery phases are the important part of the operation in the cultivation of many tropical tree plants. Keeping the seedlings growing in the nursery until they are big enough, tougher and more vigorous save seeds, space, water and reduces the risk of damage to or loss of the plant (Muhammad and Amusa 2003).

The comparative study on the effect of Normal Water, Hot water, Scarification and varying concentration of sulphuric acid (H_2SO_4) on growth performance and seed germination of *Tamarindus indica* will provide necessary information needed for the standard parameters in breaking the seed dormancy in order to increase the production of desired plants raw materials by raising a plants with desired characteristics, these will help in developing the fastest and suitable methods of breaking the seed dormancy and conservations for industrialization purpose in order to minimize the rate of extinction of *Tamarindus indica* as well as to enhance its utilization efficiency which may encourage its productivity and growth performance. Furthermore, there is the need to promote sustainable agricultural and conservation practices to ensure that *Tamarindus indica* and other plants species can continue to thrive and provide ecological and economic benefits as well as to satisfy the needs for the raw materials in the pharmaceutical industries for the next generation to come.

MATERIALS AND METHODS

Sample Collection:

Seven hundred (700) seeds of *Tamarindus indica* were obtained from College of Agricultural Science (CAS) Mando Area of Kaduna State, Nigeria. The seeds were collected using a random sampling technique (RST) from the tree stand after the fruit fall. The seeds were identified and authenticated at Botany Department of Nigerian Defence Academy, Kaduna. Cow dung was collected from Millenium City Danbushiya village and sand and top soil from biological garden, NDA Kaduna.

Research Design.

The research work comprised of laboratory tests of dormancy breakage using germination incubator machine and other laboratory activities. Four (4) different treatments were used in breaking the seed dormancy of *Tamarindus indica* in this study i.e. Normal water, Hot water, Scarification Methods and Sulphuric acid Treatments Method. Proximate analysis was also carried out which comprises ash content, Moisture contents, Proteins content, Fats and oil, carbohydrates, and Crude Fiber. Germination assessment consists of 70 observation plots (Petri dishes) and each contains at least 10 viable seeds of the study species. Similarly, 20 observation plots of each treatment (polythene bags containing potting mixtures of sand and cow dung) were used for early growth assessment, with 10 set of control.

Seed Processing

The Seeds samples of *Tamarindus indica* were soaked in water overnight. The fleshy fruits were removed and the seed were extracted and air dried for over 6 hours. The damaged seeds were sorted out from the good ones and discarded. Some seeds were bigger in size than the others. Also, the seeds have different shapes and they were tagged according to their shapes. All experiments were conducted at the botany laboratory Nigerian Defence Academy and Biochemistry Department of Kaduna state university under laboratory conditions.

Test for Seeds Viability

Viability tests; these include scarification, cutting test, excised embryo and floatation methods. Floatation methods were adopted because it is the fastest way of testing seeds viability, which is based on the observation that empty or nonviable seeds float while viable seeds sink or settle down to the bottom of the container. In this experiment, 150 seeds of *Tamarindus indica* of each treatment that is 700 seeds; each were soaked into a 400 mL beaker containing water and observed for 10 to 15 minutes to identify the viable seeds.

Qualitative Phytochemical Analysis

The phytochemical analysis was carried out following the procedures described by Harborne, (1992). The secondary metabolites screened include:

Alkaloids

To test for alkaloid, 0.1 mg of each plant extracts were measured into 6 ml of diluted HCl. The mixtures were boiled at 85 °C, allowed to cool at 40 °C and filtered using a small 1inch sieve. The presence of alkaloids in filtrates were tested using Dragendorff's reagent, Meyer's reagent and Wagner's reagent each in separate container.

Terpenoid

In order to test for terpenoid, 1 ml of each plant extracts were dispensed into 10 ml of deionized water before adding 3 drops of ferric chloride in the solution.

Flavonoids

The flavonoid composition was determined by boiling 0.2 mg of each extract at 40°C in 10 ml of ethyl acetate for 3 minutes. It was cooled at room temperature and filtered through 1 inch sieve. Then 4 ml of the filtrate was mixed with 1ml of dilute ammonia solution and shook vigorously for about 1 min.

Saponins

Presence of saponins were determined by adding 5 ml of each plant extract into 20 ml of deionized water and shook vigorously.

Phenols

A small amount of the plant extract was taken with 1 mL of water in a test tube and 1 to 2 drops of Iron III chloride (FeCl₃) was added.

Tannins

A 0.5g of the powdered plant extract was mixed in test tube containing 20ml of distilled water. It was heated at 40°C for 5 mins, then filtered using 2 inches sieve and then 0.1 % FeCl₃ was added.

Glycosides

Glycosides was determined by adding 1 ml of concentrated H₂SO₄ to 5 ml of each of the plant extract. Afterward, 2 ml of glacial CH₃CO₂H containing 1 drop of FeCl₃ was then added to the mixture.

Quantitative Phytochemical Analysis

The phytochemicals constituents of the leaves and stem bark extracts of *Annona muricata* were determined following standard procedures as described below.

Determination of total phenols

Hundred milligram (100 mg) of the plant extracts were dissolved in 100 ml of triple distilled water (TDW). A 1 ml of the resultant solution was then transferred to a test tube, and 0.5 ml 2 N of the Folin Ciocalteu reagent was added. Afterward, 1.5 ml 20 % of Na₂CO₃ solution was then added and the volume was made up to 8 ml with TDW followed by vigorous shaking and finally allowed to stand for 2 h. The absorbance of the solution was taken at 765 nm. The values obtained was used to estimate the total phenolic content from a standard calibration curve prepared from various concentrations of gallic acid (Hagerman *et al.*, 2000).

Determination of total flavonoids

The method is based on the formation of the flavonoids - aluminium complex which has an absorptivity maximum at 415 nm. 100 µl of the sample extracts in methanol (10 mg/ml) was mixed with 100 µl of 20 % aluminum trichloride in methanol and a drop of acetic acid, and then diluted with methanol to 5 ml. The absorption at 415 nm was read after 40 minutes. Blank samples were prepared from 100 ml of sample extracts and a drop of acetic acid, and then diluted to 5 ml with methanol. The absorption of standard rutin solution (0.5 mg/ml) in methanol was measured under the same conditions. All determinations were carried out in triplicates (Kumaran and Karunakaran, 2006).

Determination of total alkaloids

Five gram (5 g) of the sample was weighed into a 250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed (Harborne, 1992).

Determination of total tannins

Five hundred (500 mg) of the samples were weighed into a 50 ml plastic bottle. 50 ml of distilled water were added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M FeCl_3 in 0.1 M HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 mins (Van-Burden and Robinson, 1981).

Determination of total saponins

Twenty gram (20 g) of the ground plant extract was weighed into conical flask and 100 cm^3 of 20 % aqueous ethanol were added. It was then heated on a hot water bath set at 55 °C for 4 h with continuous stirring. The mixture was filtered and the residue re-extracted with fresh 200 ml 20 % ethanol. The combined extracts were then evaporated to 40 ml over water bath at about 90 °C. The concentrate was transferred into a 250 ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 ml of n-butanol was added. The combined n-butanol extracts were then washed twice with 10 ml of 5 % aqueous sodium chloride and solution was evaporated in a water bath. The resultant samples were then dried in the oven to a constant weight and the saponin content calculated (Obdoni and Ochuko, 2001).

Determination of Glycosides

Cardiac glycoside content in the sample was evaluated using Buljet's reagent as described by El-Olemy (1994). 1 g of the fine powder of *Annona muricata* leaves was soaked in 10 ml of 70 % alcohol for 2 h. and then filtered. The extract obtained was then purified using lead acetate and Na_2HPO_4 solution before the addition of freshly prepared Buljet's reagent (containing 95 ml aqueous picric acid + 5 ml 10 % aqueous NaOH). The difference between the intensity of colours of the experimental and blank (distilled water and Buljet's reagent) samples gives the absorbance and is proportional to the concentration of the glycosides.

Germination Tests

Dilute Sulphuric Acid (Dil. H_2SO_4) Treatment, Hot Water Treatment, Normal Water and Scarification Treatment were used in this experiment

Preparation of Solution

The dilute sulphuric acid was prepared using dilution method of liquid reagents with the formula: $C_1V_1=C_2V_2$.

Where; V= volume, C= concentration, C_1V_1 = source solution attributes, C_2V_2 = new solution attributes. As follow:

At 10% of dilute sulphuric acid concentration, 10mL of dilute

sulphuric acid (dil. H_2SO_4) were added to 90mL of distilled water, at 20%, 20mL of dilute sulphuric acid (dil. H_2SO_4) was added to 80mL of distilled water, at 30%, 30mL of dilute sulphuric acid (dil. H_2SO_4) was added to 70mL of distilled water, at 40%, 40mL of dilute sulphuric acid (dil. H_2SO_4) was added to 60mL of distilled water, at 50%, 50mL of dilute sulphuric acid (dil. H_2SO_4) was added to 50mL of distilled water.

Sulphuric Acid (Dilute. H_2SO_4) Treatment

Dilute Sulphuric Acid (H_2SO_4) Treatment: in this experiment, 150 seeds of *Tamarindus indica* were soaked into a beaker containing 500 mL of Conc. H_2SO_4 for 30 minutes which is accompanied by stirring in order to ensure equal treatment of the seeds. The treated seeds were washed thoroughly with distilled water and were transferred to the Petri dishes containing moistened filter paper for germination assessment (Colleen, 2014).

Percentage Rate of Germination

The following varying concentrations were used for the studies. 10% for 5 minutes, 20% for 10minutes, 30% for 15minutes, 40% for 20 minutes; and 50% for 25 minutes. Control for untreated seeds (Colleen, 2014)

The seeds of *Tamarindus indica* were soaked into a beaker, distilled water was transferred into beaker using 20mL syringe. The seed treatment was done at 10%, 20%, 30%, 40% and 50%. The treatment was carried out as described below by Colleen (2014).

Seeds Treatment at 10% Dilute H_2SO_4

The seeds were added into the diluted concentrated sulphuric acid and monitored for 5 minutes for changes that will occur. The treated seeds were washed thoroughly with water and transferred to the petri dishes containing moistened filter paper for germination assessment (Colleen, 2014).

Seeds Treatment at 20% Dilute H_2SO_4

The seeds were added into the diluted concentrated sulphuric acid and monitored for 10 minutes. Which is accompanied by stirring in order to ensure equal treatment of the seeds. The treated seeds were washed thoroughly with water and transferred to the petri dishes containing moistened filter paper for germination assessment (Colleen, 2014).

Seeds Treatment at 30% Dilute H_2SO_4

The seeds were added into the diluted concentrated sulphuric acid and monitored for 15 minutes. This was accompanied by stirring in order to ensure equal treatment of the seeds. The treated seeds were washed thoroughly with water and transferred to the petri dishes containing moistened filter paper for germination assessment (Colleen, 2014).

Seeds Treatment at 40% Dilute H_2SO_4

The seeds were added into the diluted concentrated sulphuric acid and observed for 20 minutes. Which is accompanied by stirring in order to ensure equal treatment of the seeds. The treated seeds were washed thoroughly with water and transferred to the petri dishes containing moistened filter paper for germination assessment (Colleen, 2014).

Seeds Treatment at 50% Dilute H_2SO_4

The seeds were added into the diluted concentrated sulphuric acid and were monitored for 25 minutes. Which is accompanied by

stirring in order to ensure equal treatment of the seeds. The treated seeds were washed thoroughly with water and transferred to the petri dishes containing moistened filter paper for germination assessment (Colleen, 2014).

Hot Water Treatment

A 400 mL beaker was filled with water and heated to a temperature of 100°C using electric heater. One hundred and Fifty (150) viable seeds were soaked into the boiling water, and the heat source was immediately removed; the seeds were allowed to settle in cooling water for a period of one hour and then transferred to the petri dishes containing moistened filter paper for germination assessment.

Normal Water Treatment

Similarly in this trial 150 viable seeds were soaked in 400 mL beaker containing 400 mL of water (at room temperature) for 12 hrs. The seeds were removed and transferred to the petri dishes containing a moistened filter paper to examine the seed germination.

Scarification Method

In this method, the seeds coat of *Tamarindus indica* were treated with sulphuric acid for few minutes and then followed by scarifying the seeds for about 1 minute on a cleaned dry place and thoroughly cleaned in water.

Patterns of Different Chemicals Mobilization in the Germinating *Tamarindus indica* Seed

Seed germination is applied to the resumption of the growth of seed's embryo after the period of dormancy, therefore breaking of seed dormancy prompts the seed to release these chemicals rapidly. Study on the mobilization patterns of chemicals during

seed germination was carried out to ensure the successful growth and development of new plants. By monitoring and optimizing these patterns, solubility, volatility, and other physicochemical properties such as water-soluble and other chemicals may be more easily mobilized during germination than hydrophobic chemicals, the study on these chemicals, their mobilization and their enzymatic activity of breaking the seed dormancy during germination process was conducted. Study on the mobilized chemicals provided the information on the chemicals that facilitate initial burst of seeds and the growth of new seedlings.

Data Analysis

The data was presented as mean \pm standard error of mean (SEM). Analysis of variance (ANOVA) and visualising data in SPSS version 23.0 were used for statistical analysis, and values with $p \leq 0.05$ were chosen for statistical significance difference.

RESULTS

The Percentage Yield of *Tamarindus indica* seeds Extract

The percentage yield of *Tamarindus indica* seeds were approximately 33%, 31.3%, 12.7% and 7.6% for hot water treatment at day 0, day 5, day 10 and day 15 respectively. Normal water treatment was approximately 32.3%, 27%, 10% and 6.3% at day 0, day 5, day 10 and day 15 respectively. After Scarification, the percentage yield was approximately 27.7%, 23%, 6.33% and 3% at day 0, day 5, day 10 and day 15 respectively. The percentage yield after sulphuric acid treatment was approximately 21.7%, 17%, 2.7% and 2.3% at day 0, day 5, day 10 and day 15 respectively, while the control shows a higher percentage yield of approximately 43%, 34.7%, 18% and 9.7% at day 0, day 5, day 10 and day 15 respectively as shown in Table 1.

Table 1: The Percentage Yield of *Tamarindus indica* seeds Extract

| Sample | Day 0 | Day 5 | Day 10 | Day 15 |
|----------------|-------|-------|--------|--------|
| Hot Water | 38.0% | 31.3% | 12.7% | 7.6% |
| Normal Water | 32.3% | 27.0% | 10.0% | 6.3% |
| Scarification | 27.7% | 23.0% | 6.33% | 3.0% |
| Sulphuric Acid | 21.7% | 17.0% | 2.7% | 2.3% |
| Control | 43.0% | 34.7% | 18.0% | 9.7% |

Phytochemical Analysis of *Tamarindus indica*

The result of qualitative and quantitative phytochemical analysis of the leaf extract of *Tamarindus indica* is presented in Tables 2a, 2b, 2c and 2d. The qualitative analysis of the seeds showed the presence of alkaloids, flavonoids, saponins, phenols, tannins and

glycosides and steroids after treatment with hot water, normal water, scarification, sulphuric acid and control at day 0, day 5, day 10 and day 15 while only glycosides were absent at day 15.

Table 2a: Qualitative Phytochemical Analysis of *Tamarindus indica* Seed at Day 0

| Phytochemical Compounds | Hot water | Normal Water | Scarification | Sulphuric Acid | Control |
|-------------------------|-----------|--------------|---------------|----------------|---------|
| Alkaloids | + | + | + | + | + |
| Steroids | + | + | + | + | + |
| Flavonoids | + | + | + | + | + |
| Saponins | + | + | + | + | + |
| Phenols | + | + | + | + | + |
| Tannins | + | + | + | + | + |
| Glycosides | + | + | + | + | + |

Table 2b: Qualitative Phytochemical Analysis of *Tamarindus indica* Seed at Day 5

| Phytochemical Compounds | Hot water | Normal Water | Scarification | Sulphuric Acid | Control |
|-------------------------|-----------|--------------|---------------|----------------|---------|
| Alkaloids | + | + | + | + | + |
| Steroids | + | + | + | + | + |
| Flavonoids | + | + | + | + | + |
| Saponins | + | + | + | + | + |
| Phenols | + | + | + | + | + |
| Tannins | + | + | + | + | + |
| Glycosides | + | + | + | + | + |

Table 2c: Qualitative Phytochemical Analysis of *Tamarindus indica* Seed at Day 10

| Phytochemical Compounds | Hot water | Normal Water | Scarification | Sulphuric Acid | Control |
|-------------------------|-----------|--------------|---------------|----------------|---------|
| Alkaloids | + | + | + | + | + |
| Steroids | + | + | + | + | + |
| Flavonoids | + | + | + | + | + |
| Saponins | + | + | + | + | + |
| Phenols | + | + | + | + | + |
| Tannins | + | + | + | + | + |
| Glycosides | + | + | + | + | + |

Table 2d: Qualitative Phytochemical Analysis of *Tamarindus indica* Seed at Day 15

| Phytochemical Compounds | Hot water | Normal Water | Scarification | Sulphuric Acid | Control |
|-------------------------|-----------|--------------|---------------|----------------|---------|
| Alkaloids | + | + | + | + | + |
| Steroids | + | + | + | + | + |
| Flavonoids | + | + | + | + | + |
| Saponins | + | + | + | + | + |
| Phenols | + | + | + | + | + |
| Tannins | + | + | + | + | + |
| Glycosides | - | - | - | - | - |

The quantitative analysis of the seeds has indicated that there were no statistical differences in the total alkaloids content (mgAE/g) at all days. Statistical significance differences were recorded for total flavonoids content (mgQE/g) at day 15. However there were statistical differences in the total phenolic content (mgGAE/g) between all the groups at all days with the exception of day 10

which does not show statistical significance between the groups. There was statistical significance differences recorded for both the total saponins (mgDE/g) and total tannins (mgDE/g) at day 15 between all the groups, while no significance differences were recorded at day 0, day 5 and day 10 between all the groups as shown in Tables 3a, 3b, 3c, 3d and 3e respectively.

Table 3a: Determination of Total Alkaloids (mgAE/g) in *Tamarindus indica* Seeds

| GROUPS | DAY 0 | DAY 5 | DAY 10 | DAY 15 |
|---------------|---------------|---------------|----------------|---------------|
| Sulfuric acid | 29.987±14.120 | 63.563±9.620 | 16.983±7.960 | 33.173±22.665 |
| Scarification | 45.130 ±7.263 | 74.073±10.265 | 18.407±6.752 | 83.960±18.912 |
| Hot water | 39.917±9.609 | 73.847±14.608 | 13.303±4.245 | 4.127±1.553 |
| Normal water | 56.517±11.577 | 72.177±3.450 | 8.633±4.534 | 78.330±20.335 |
| Control | 34.923±23.056 | 57.860±17.267 | 24.2767±25.094 | 6.533±2.419 |

Values are expressed as mean ± SEM. Statistical significance mean difference was considered at p<0.05 and Duncan comparison test was used for post hoc analysis. Values bearing same superscripts under the same column are significantly different.

Table 3b: Determination of Total Flavonoids (mgQE/g) in *Tamarindus indica* Seeds

| GROUPS | DAY 0 | DAY 5 | DAY 10 | DAY 15 |
|---------------|----------------|----------------|---------------|----------------|
| Sulfuric acid | 26.987 ±11.121 | 60.563±9.623 | 14.983±8.960 | 32.173±21.677* |
| Scarification | 42.130±4.261 | 70.073±10.264 | 16.407±5.753 | 81.960±15.111* |
| Hot water | 35.917±6.608 | 71.847 ±14.681 | 11.303±4.245 | 7.127±1.533* |
| Normal water | 55.517±10.576 | 72.177±3.450 | 10.633±3.534 | 76.330±16.344* |
| Control | 31.923±20.054 | 55.860±17.266 | 22.277±19.094 | 9.533±0.400* |

Values are expressed as mean \pm SEM. Statistical significance mean difference was considered at $p < 0.05$ and Duncan comparison test was used for post hoc analysis. Values bearing same superscripts under the same column are significantly different.

Table 3c: Determination of Total Phenols (mgGAE/g) in *Tamarindus indica* Seeds

| GROUPS | DAY 0 | DAY 5 | DAY 10 | DAY 15 |
|---------------|--------------------|--------------------|-------------------|--------------------|
| Sulfuric acid | 5.060 \pm 0.020* | 5.120 \pm 0.000* | 5.403 \pm 0.091 | 5.403 \pm 0.091* |
| Scarification | 5.030 \pm 0.017* | 5.343 \pm 0.110* | 5.133 \pm 0.012 | 5.133 \pm 0.012* |
| Hot water | 5.097 \pm 0.006* | 5.250 \pm 0.000* | 5.450 \pm 0.017 | 5.450 \pm 0.017* |
| Normal water | 4.523 \pm 0.086* | 4.823 \pm 0.075* | 4.913 \pm 0.081 | 4.913 \pm 0.081* |
| Control | 4.780 \pm 0.193* | 5.363 \pm 0.098* | 5.293 \pm 0.150 | 5.293 \pm 0.150* |

Values are expressed as mean \pm SEM. Statistical significance mean difference was considered at $p < 0.05$ and Duncan comparison test was used for post hoc analysis. Values bearing same superscripts under the same column are significantly different.

Table 3d: Determination of Total Saponins (mgDE/g) in *Tamarindus indica* Seeds

| GROUPS | DAY 0 | DAY 5 | DAY 10 | DAY 15 |
|---------------|---------------------|---------------------|---------------------|----------------------|
| Sulfuric acid | 23.987 \pm 10.120 | 58.563 \pm 4.620 | 15.983 \pm 4.973 | 29.173 \pm 22.555* |
| Scarification | 39.130 \pm 2.263 | 71.073 \pm 8.265 | 17.407 \pm 5.749 | 78.960 \pm 18.222* |
| Hot water | 32.917 \pm 7.609 | 68.847 \pm 13.608 | 12.303 \pm 1.241 | 6.127 \pm 1.543* |
| Normal water | 50.517 \pm 8.577 | 67.177 \pm 0.450 | 6.633 \pm 3.530 | 69.330 \pm 20.432* |
| Control | 32.923 \pm 11.056 | 51.860 \pm 12.267 | 20.200 \pm 22.139 | 4.533 \pm 2.786* |

Values are expressed as mean \pm SEM. Statistical significance mean difference was considered at $p < 0.05$ and Duncan comparison test was used for post hoc analysis. Values bearing same superscripts under the same column are significantly different.

Table 3e: Determination of Total Tannins (mgDE/g) in *Tamarindus indica* Seeds

| Groups | DAY 0 | DAY 5 | DAY 10 | DAY 15 |
|---------------|---------------------|---------------------|---------------------|----------------------|
| Sulfuric acid | 30.980 \pm 11.121 | 61.435 \pm 6.333 | 11.988 \pm 7.966 | 32.177 \pm 19.135* |
| Scarification | 40.121 \pm 5.233 | 72.070 \pm 9.244 | 14.422 \pm 5.356 | 80.961 \pm 12.421* |
| Hot water | 38.806 \pm 9.598 | 72.736 \pm 13.597 | 12.312 \pm 3.234 | 3.116 \pm 1.000* |
| Normal water | 55.416 \pm 10.466 | 69.165 \pm 2.341 | 7.532 \pm 3.433 | 76.312 \pm 17.114* |
| Control | 31.819 \pm 19.044 | 53.751 \pm 16.156 | 21.165 \pm 14.081 | 4.411 \pm 1.322* |

Values are expressed as mean \pm SEM. Statistical significance mean difference was considered at $p < 0.05$ and Duncan comparison test was used for post hoc analysis. Values bearing same superscripts under the same column are significantly different.

Germination of Seed after Treatment

The germination percentage of *Tamarindus indica* treated normal water, hot water, scarification, sulphuric acid and control revealed significant differences between treatments. Because scarification of the seed coupled with acid and boiling water treatments improved germination of the seeds faster, this can infer that dormancy of their seeds is physical and is related to the hard coat of the seeds. The seed coat is a physical barrier against growth of the embryo or radicle, which also inhibits absorption of water and gas exchange. The results on number of seeds germinated, number of leaves and plants' height of both the dark brown, normal brown, and light brown seeds were presented in Tables 5a, 5b, 5c,

5d and 5e. The Results revealed highly significant difference between the different treatments on germination rate. The highest germination rate, was observed in mechanically scarified, normally and acidified seeds followed by those treated with hot water. The untreated seeds (control) recorded was the lowest. The result of different percentage concentration of sulphuric acid shows significance differences across the different concentrations of 10%, 20%, 30%, 40% and 50% with the highest number of seeds germinated, number leaves and plant height, followed observed at 50%, then followed by 40%, 30%, 20% and 10% displacing the least as shown in Tables 5f and 5g.

Table 5a: Effect of Normal water Treatment on Germination of *Tamarindus indica* Seeds

| Number of Days | Dark Brown | | | Normal Brown | | | Light Brown | | |
|----------------|------------|----|----|--------------|----|----|-------------|----|----|
| | NGS | NL | PH | NGS | NL | PH | NGS | NL | PH |
| 7 | - | - | - | - | - | - | - | - | - |
| 11 | - | - | - | - | - | - | - | - | - |
| 17 | 1 | - | - | 4 | - | - | 2 | - | - |
| 22 | 3 | - | - | 4 | 2 | 2 | 2 | - | - |
| 24 | 2 | - | - | 5 | 3 | 2 | 2 | - | - |
| 26 | 2 | 1 | 2 | 8 | 4 | 3 | 2 | 1 | 2 |
| 28 | 4 | 2 | 2 | 10 | 6 | 4 | 2 | 1 | 2 |
| 30 | 6 | 3 | 2 | 15 | 8 | 5 | 4 | 2 | 2 |
| 34 | 8 | 4 | 3 | 20 | 10 | 7 | 6 | 3 | 3 |
| 36 | 10 | 6 | 4 | 25 | 10 | 8 | 8 | 4 | 4 |
| 40 | 15 | 10 | 6 | 30 | 12 | 10 | 10 | 6 | 6 |
| 45 | 20 | 12 | 10 | 40 | 16 | 13 | 16 | 8 | 10 |
| 50 | 32 | 14 | 13 | 50 | 16 | 16 | 20 | 10 | 12 |

Key:

NGS = Number of germinated seeds;
 NL = Number of leaves;
 PH = Plant height

Table 5b: Effect of Hot water Treatment on Germination of *Tamarindus indica* Seeds

| Number of Days | Dark Brown | | | Normal Brown | | | Light Brown | | |
|----------------|------------|----|----|--------------|----|----|-------------|----|----|
| | NGS | NL | PH | NGS | NL | PH | NGS | NL | PH |
| 7 | 2 | - | - | 2 | - | - | - | - | - |
| 11 | 3 | - | - | 3 | - | - | 4 | - | - |
| 17 | 11 | - | - | 15 | - | - | 10 | - | - |
| 22 | 30 | 1 | 2 | 35 | 1 | 2 | 29 | 1 | 2 |
| 24 | 35 | 4 | 4 | 38 | 3 | 4 | 32 | 2 | 4 |
| 26 | 38 | 6 | 11 | 40 | 5 | 6 | 35 | 4 | 5 |
| 28 | 40 | 10 | 13 | 45 | 12 | 14 | 38 | 8 | 10 |
| 30 | 40 | 14 | 15 | 48 | 16 | 16 | 40 | 10 | 12 |
| 34 | 45 | 14 | 16 | 50 | 16 | 16 | 43 | 12 | 14 |
| 36 | 45 | 14 | 16 | 50 | 16 | 16 | 45 | 14 | 14 |
| 40 | 48 | 16 | 16 | 50 | 16 | 16 | 48 | 14 | 16 |
| 45 | 50 | 16 | 16 | 50 | 16 | 16 | 50 | 16 | 16 |
| 50 | 50 | 16 | 16 | 50 | 16 | 16 | 50 | 16 | 16 |

Key:

NGS = Number of germinated seeds;
 NL = Number of leaves;
 PH = Plant height

Table 5c: Effect of Scarification Treatment on Germination of *Tamarindus indica* Seeds

| Number of Days | Dark Brown | | | Normal Brown | | | Light Brown | | |
|----------------|------------|----|----|--------------|----|----|-------------|----|----|
| | NGS | NL | PH | NGS | NL | PH | NGS | NL | PH |
| 7 | 10 | - | - | 10 | - | - | 10 | - | - |
| 11 | 18 | - | - | 20 | - | - | 15 | - | - |
| 17 | 22 | - | - | 25 | - | - | 20 | - | - |
| 22 | 30 | 10 | 3 | 30 | 10 | 4 | 25 | 10 | 2 |
| 26 | 30 | 10 | 5 | 35 | 12 | 10 | 27 | 10 | 7 |
| 28 | 37 | 11 | 9 | 40 | 12 | 12 | 30 | 10 | 7 |
| 30 | 40 | 12 | 10 | 40 | 14 | 14 | 35 | 10 | 7 |
| 34 | 42 | 13 | 13 | 45 | 14 | 14 | 38 | 10 | 11 |
| 36 | 42 | 14 | 13 | 50 | 14 | 16 | 43 | 12 | 15 |
| 40 | 48 | 14 | 15 | 50 | 16 | 16 | 48 | 12 | 15 |
| 45 | 50 | 16 | 16 | 50 | 16 | 16 | 50 | 14 | 16 |
| 50 | 50 | 16 | 16 | 50 | 16 | 16 | 50 | 16 | 16 |

Key:

NGS = Number of germinated seeds;
 NL = Number of leaves; PH = Plant height

Table 5d: Effect of Control Treatment on Germination of *Tamarindus indica* Seeds

| Number of Days | Dark Brown | | | Normal Brown | | | Light Brown | | |
|----------------|------------|----|----|--------------|----|----|-------------|----|----|
| | NGS | NL | PH | NGS | NL | PH | NGS | NL | PH |
| 7 | - | - | - | - | - | - | - | - | - |
| 11 | - | - | - | - | - | - | - | - | - |
| 17 | - | - | - | 2 | - | - | - | - | - |
| 22 | 1 | - | - | 4 | - | - | - | - | - |
| 24 | 1 | - | - | 4 | - | - | 2 | 1 | - |
| 26 | 1 | - | - | 4 | 3 | 2 | 2 | 1 | 2 |
| 28 | 3 | 2 | 2 | 10 | 14 | 6 | 3 | 2 | 2 |
| 30 | 3 | 10 | 8 | 19 | 14 | 10 | 3 | 4 | 4 |
| 34 | 8 | 12 | 10 | 27 | 16 | 13 | 5 | 8 | 8 |
| 36 | 10 | 12 | 10 | 32 | 16 | 14 | 10 | 10 | 10 |
| 40 | 15 | 13 | 11 | 40 | 16 | 16 | 12 | 12 | 10 |
| 45 | 20 | 15 | 13 | 48 | 16 | 16 | 15 | 14 | 12 |
| 50 | 28 | 16 | 16 | 50 | 16 | 16 | 20 | 16 | 16 |

Key:

NGS = Number of germinated seeds;
 NL = Number of leaves;
 PH = Plant height

Table 5e: Effect of Sulphuric acid Treatment on Germination of *Tamarindus indica* Seeds

| Number of Days | Dark Brown | | | Normal Brown | | | Light Brown | | |
|----------------|------------|----|----|--------------|----|----|-------------|----|----|
| | NGS | NL | PH | NGS | NL | PH | NGS | NL | PH |
| 7 | 10 | - | - | 4 | - | - | 1 | - | - |
| 11 | 10 | - | - | 10 | - | - | 2 | - | - |
| 17 | 12 | - | - | 15 | - | - | 10 | - | - |
| 22 | 18 | 6 | 4 | 20 | 10 | 10 | 15 | 4 | 2 |
| 26 | 25 | 6 | 6 | 29 | 10 | 10 | 18 | 6 | 4 |
| 28 | 30 | 8 | 6 | 35 | 12 | 10 | 23 | 8 | 6 |
| 30 | 35 | 8 | 8 | 41 | 12 | 12 | 27 | 10 | 6 |
| 34 | 39 | 10 | 10 | 47 | 16 | 14 | 32 | 10 | 8 |
| 36 | 43 | 12 | 10 | 50 | 16 | 16 | 37 | 12 | 10 |
| 40 | 47 | 14 | 12 | 50 | 16 | 16 | 40 | 14 | 12 |
| 45 | 50 | 16 | 14 | 50 | 16 | 16 | 43 | 16 | 16 |
| 50 | 50 | 16 | 16 | 50 | 16 | 16 | 50 | 16 | 16 |

Key:

NGS = Number of germinated seeds;
 NL = Number of leaves;
 PH = Plant height

Table 5f: Effect of Sulphuric acid Percentage Concentration Treatment on Germination of *Tamarindus indica* Seeds

| Number of days | Number of Germinated Seeds | | | | | |
|----------------|----------------------------|-----|-----|-----|-----|----|
| | 10% | 20% | 30% | 40% | 50% | |
| 7 | 3 | | 1 | 4 | 4 | 7 |
| 13 | 4 | | 6 | 8 | 8 | 10 |
| 15 | 8 | | 10 | 14 | 14 | 15 |
| 17 | 15 | | 19 | 23 | 24 | 27 |
| 22 | 25 | | 29 | 33 | 34 | 37 |
| 25 | 27 | | 31 | 35 | 36 | 40 |
| 30 | 29 | | 32 | 35 | 38 | 40 |
| 35 | 30 | | 33 | 35 | 39 | 45 |
| 40 | 35 | | 37 | 37 | 41 | 48 |
| 45 | 40 | | 40 | 41 | 45 | 50 |
| 48 | 45 | | 45 | 47 | 48 | 50 |
| 50 | 45 | | 45 | 48 | 48 | 50 |

Table 5g: Effect of Sulphuric acid Percentage Concentration Treatment on Number of leaves and Plant height

| Number of days | Plant Height/Number of Leaves | | | | | |
|----------------|-------------------------------|-------|-----|-------|-------|-------|
| | 10% | 20% | 30% | 40% | 50% | |
| 15 | 8/4 | 10/4 | | 11/8 | 11/6 | 10/16 |
| 17 | 9/9 | 11/11 | | 12/12 | 12/12 | 13/18 |
| 22 | 15/14 | 20/15 | | 20/15 | 23/15 | 20/31 |
| 25 | 16/24 | 20/25 | | 22/25 | 25/35 | 35/38 |
| 30 | 18/25 | 20/26 | | 24/27 | 26/36 | 37/39 |
| 35 | 18/27 | 22/27 | | 24/28 | 26/37 | 37/40 |
| 40 | 25/29 | 26/30 | | 27/30 | 29/39 | 39/44 |
| 45 | 25/31 | 27/31 | | 29/33 | 29/43 | 40/44 |
| 48 | 25/34 | 30/35 | | 31/38 | 33/45 | 42/48 |

DISCUSSION

The Tamarind indica seed's phytochemical screening also reveals the presence of steroids, phenols, saponins, glycosides, tannins, and flavonoids. The plants' phytochemical screening provides a general understanding of the class of compounds those plants contain. Chemical tests are necessary to determine the different elements or groups present in the plants, even though the usefulness of plants mostly rely on their active principles that are therapeutically beneficial (Sinha *et al.*, 2017). It is quite probable that the antioxidant and anti-inflammatory qualities stem from the presence of tannins. Other therapeutic effects are attributed to flavonoids. The role that phytochemicals play in plants may help us better understand the ways in which they assist people (Mujeeb *et al.*, 2014).

This study showed how well sulfuric acid pretreatments work to thaw *Tamarind indica* dormancy. This result confirms the finding of (Duguma *et al.*, 1988) that the most important and effective method for increasing *Leucaena* seed coat permeability is sulfuric acid scarification. Other plants that have been reported to have it include *Tamarindus indica* (Muhammad and Amusa, 2003), *Prosopis koelziana* (Agbogidi *et al.*, 2007), *Leucaena diversifolia* (Dos Santos Carvalho *et al.*, 2007), *Prosopis juliflora* (Zare *et al.*, 2011), *Centrosema pubescens* (Rusdy *et al.*, 2015), *L. Leucaena* (Rusdy, 2017), and *Adenantha pavonina* (Mantoan *et al.*, 2012). These are just a few instances of plants that have reportedly been found to possess it. Since impermeability has been linked to the degree of impermeability in several species, the majority of research conclude that the cuticle is the site of thickness (Yousif *et al.*, 2020). It is thought that sulfuric acid disrupts the seed coat and the palisade epidermal layer. This causes water to be absorbed, which triggers the release of less sugar that can be utilized for protein synthesis and promotes germination. Additionally, the results indicate that acid scarification is more successful than hot water in reducing germination in *Leucaena* seeds. This is especially true for seeds that were exposed to hot water for an extended period of time, which likely killed the embryo and reduced germination when compared to sulfuric acid treatment. Consequently, most of the seeds treated with sulfuric acid for 17, 10, and 34 days showed germination, indicating that the method was successful in preventing the seeds from going dormant (Teles *et al.*, 2000).

According to a number of studies (Baskin *et al.*, 2000; Baskin, 2003; Ma *et al.*, 2004), the major lignin current in the epidermal palisade-like tissue is often what causes the seed coat impermeability. According to Kelly *et al.* (1992), there is evidence

in multiple species that variations in the degree of lignification of palisade-like layers can cause some seeds to be both non-permeable and water-permeable. Consequently, Baskin *et al.* (2000) suggested that even though the cells in the seed coat were lignified, the seed should still be water-tight due to the simple facing of a palisade-like tissue layer. According to Delouche (2000), the hard seed coat's water resistance is the primary factor causing Fabaceae seed dormancy. Gupta *et al.* (2001) and Ghadiri and Torshiz (2000) both reported similar outcomes when working with *Psoralea corilifolia* and *Glycyrrhiza glabra* L. This corroborates the findings of Hartmann and Kester's (1995) study, which showed that seeds can be effectively mechanically, chemically, and physically dormant by scarification. The number of days needed for treated seeds to germinate decreased, according to the data. According to Souza and Silva (1998), mechanical scarification resulted in the highest rates of seedling emergence and germination. The shortened time to germination could potentially be attributed to the seed coat's increased susceptibility to water through perforations in the tough outer layer. This result is consistent with that of Mayer and Poljakoff-Mayber (1989), who found that when the seed coat is torn or punctured by mechanical abrasion or chemical treatment, even the hardest-coated seeds become water-permeable. Muhammad and Amusa (2003) found that treating tamarind (also known as "Jabbe") seeds with 50% sulfuric acid for 60 minutes produced a comparable 98% germination rate. According to Wang (2007), the majority of pretreatments dramatically lower the hard seed content while increasing the germination rate and growth rate.

According to Nikolaeva (2007), *P. pinnata* treated with hot water for 15 minutes increased seedling emergence. Additionally, it was discovered that soaking seeds in 80°C water for 24 hours after pouring the water over them in a container worked well for *Acacia nilotica* and *Tamarindus indica*. It has been observed that soaking seeds of *Adansonia digitata*, *Calliandra calothyrsus*, and *Sesbania sesban* in water at 100°C for 24 hours, followed by continuous soaking, effectively breaks seed coat dormancy and promotes seedling emergence (Albrecht, 1993). Additionally, Pasiecznik *et al.* (2009) observed that boiling water treatment of *Prosopis* species seeds resulted in an increase in seedling emergence. The reduction in the time needed for treated seeds to germinate could be caused by boiling water rupturing and shattering the testa's outer layer, which would increase the seed coat's water permeability. This finding corroborates the findings of Kannan *et al.* (2001), who observed that heating seeds to 100 degrees Celsius during dormancy breaking caused alterations in the seed coat structure, which in turn caused the testa to rupture close to the hilum. The length of soaking time affects the number of days to

germination; the longer the soaking time, the fewer days to germination. This also aligns with the results of Saikou *et al.* (2008) on the effect of some seed pre-treatment on emergence of *Acacia senegalensis* and *Tamarindus indica*. Cell division, the enlargement of the newly formed cells, and their differentiation into various tissues result in growth in plants. A permanent change in size is a result of these growth processes (typically an increase in the growing portions such as the shoot). During the data collection process, the more straightforward approach of measuring growth (the direct method) was employed. This involved measuring the length of the growing portion (the shoot) every day after the seedlings emerged using only a centimeter ruler. According to the results, there were notable differences between the treatments in terms of shoot height at various weeks following planting. The number of days following planting did not significantly affect the number of seedlings. Awodola (1994) achieved a comparable outcome in his *Tamarindus indica* seed experiment, wherein the mechanical treatment considerably increased the plant's height. Muhammad and Amusa (2003) also had a comparable outcome in their tamarind research. Analogous research by Masamba (2010), Rungu (2006), Bonner (2009), Phartya *et al.* (2005), and others support the results of seedling height in *Delonix regia*. Similar findings have also been reported in previous research (Lima, 2008; Teketay, 2011; Pearman, 2002; Baskin *et al.*, 2004; Bamel *et al.*, 2007; Cook 2008) about the boiling water treatment for *Delonix regia* which corroborates with the findings of this research.

Conclusion

The phytochemical screening of *Tamarindus indica* seeds reveals the presence of diverse bioactive compounds, including steroids, phenols, saponins, glycosides, tannins, and flavonoids. These compounds are linked to the plant's antioxidant, anti-inflammatory, and other therapeutic properties, highlighting the importance of phytochemical analysis for understanding both plant function and potential health benefits. In terms of seed dormancy and germination, both chemical (sulfuric acid) and physical (hot water, mechanical) scarification methods have been shown to effectively break seed dormancy by disrupting the impermeable seed coat, thus enhancing water uptake and promoting germination. Among these, sulfuric acid pretreatment is consistently more effective, yielding higher and faster germination rates in *Tamarindus indica* and other leguminous species. This is attributed to the acid's ability to alter the seed coat structure, particularly the lignified palisade layer, which is a primary barrier to water permeability. Hot water treatments can also be effective but may reduce germination if exposure is excessive, potentially damaging the embryo. Integrating phytochemical knowledge with effective dormancy-breaking techniques supports both the medicinal use and propagation of *Tamarindus indica*, providing valuable insights for agriculture, forestry, and pharmacology.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-sectors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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